VOL. 3 NO. 2

BULLETIN

OF THE

TORREY BOTANICAL CLUB

FEBRUARY, 1904

The influence of carbon monoxide and other gases upon plants*

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Some time since the authors began an investigation of the influence of carbon monoxide upon plants, in which this gas was used to replace partially or entirely the nitrogen of the air. Attention was directed chiefly to the effects upon the cell-contents, upon growth and developmental phenomena and no consideration was given to the possibility of the use or absorption of the substance by the plant. Later comparative series of experiments were made with nitrous oxide and various mixtures furnished for illuminating purposes.

The striking character of the results already obtained, as well as the recent publication by Bottomley and Jackson of their conclusions as to the ability of the green plant to utilize carbon monoxide in the construction of food-material (Proc. Roy. Soc. **72**: 130. 31 Jl 1903), led the authors to make public at this time some of the important facts that have been brought to light in their work.

In most of these experiments the CO used was prepared by the decomposition of oxalic acid with strong sulphuric acid. As a check, however, some additional series have been tried with the gas prepared from other sources, namely, potassic ferro-cyanide, which when treated with concentrated sulphuric yields CO as the only gaseous product, and by passing a current of carbon dioxide over glowing charcoal, giving pure CO. Although in all cases

* Read before the Botanical Society of America at St. Louis, December 30, 1903.

[The preceding number of the BULLETIN, Vol. 31, No. 1, for January, 1904 (**31**: 1–56) was issued 19 Ja 1904.]

the chemicals used were of the purest form obtainable, it was not thought advisable to trust to the product being free from impurities. Of course in preparing CO from oxalic acid it was necessary to wash out the CO_2, which may easily be done, but in addition to this in all but the preliminary experiments the gas was passed over potassium permanganate, mercuric chloride, etc. The analyses of gases produced by both methods showed the presence of no impurity except a small portion of atmospheric air, which varied from 1.3 per cent. to 9 per cent. according to the method of preparation and the setting up of the experiment. The illuminating gas used was taken directly from the mains at Barnard College and at the New York Botanical Garden. No thorough analysis of this mixture was made, but the tests applied showed the presence of an amount of carbon monoxide not far from 40 per cent. of the total volume.

A small number of tests were made upon germinating seedlings and it was found that a proportion of 90 per cent. or higher of carbon monoxide in the air around the seeds prevented all action of swollen seeds except in the pea, which succeeded in making roots less than a centimeter in length, and ultimately killed all the seeds. A smaller proportion of the illuminating gas mixture was found to be inhibitory and fatal. Swollen seedlings of several species germinated in atmospheres containing about 70 per cent. of carbon monoxide, but the consequent growth and development was restricted.

The results obtained from the exposure of seedlings germinated in the usual manner gave many facts of great interest and significance. The seeds were placed in moist sphagnum and allowed to remain until the seedlings began to free themselves from the seedcoats, in short until the seedling reached a period of rapid growth when there would be likelihood of quick reactions to abnormal conditions. Glass vessels, varying in size according to the size of the seedlings, were then taken and after the flower-pots had been properly arranged in them, the air was completely replaced with water, after which a mixture of the gases desired was run in to displace the water, except for a water seal at the bottom. In the water some potash was placed, to absorb the CO_2 given off, great care being taken to have the flower-pot containing the seedlings

so supported that there would be no danger of the potash "crawling" and reaching the young plants. The diminution in volume caused by the absorption of CO_2 was corrected by running in pure oxygen at intervals as required — usually twice every twenty-four hours. Thus the atmosphere (as repeated analyses showed) was kept at normal oxygen pressure and the CO, being so very slightly soluble in water, did not diminish perceptibly. The same is also true of the illuminating gas experiments. In all respects, even to the mechanical manipulation, the controls were treated in the same way as the experiments with the gases. All of the plants had equal illumination, and while of course in the winter time some etiolation is unavoidable, the conditions of the bell-jars were precisely similar in any one experiment.

A list of the seedlings on which experiments have been completed, up to date, is as follows: *Vicia faba*, *Zea Mays*, *Sinapis alba*, *Helianthus annuus*, *Triticum vulgare*, *Fagopyrum Fagopyrum* and *Oryza sativa*.

The effect of these gases on the condition and growth of the above-named seedlings may be considered under the following heads: (1) Growth in length; (2) growth of secondary members; (3) growth in thickness (primary); (4) growth curvatures; (5) formation of chlorophyl. It is to be understood that in all of the experiments of this series the nitrogen of the air was replaced with either CO or illuminating gas (referred to hereafter merely as "gas").

Under the first head we may take up for consideration a considerable number of measurements which have been made. These measurements are of the length of the primary root and of the shoot, or in the case of the monocotyledonous forms of the distance from the seed to the tip of the longest leaf. There is shown in general to be a very clearly marked retardation, which must finally amount to a toxic effect, in both CO and gas. Taking the average of three experiments with *Vicia*, each experiment being in itself an average of from 8–10 individuals, we find the approximate ratio of the length of the shoot about as follows: The normal being 25, in CO it is 15, and in gas only 10; while a similar comparison of the length of the primary root gives the following ratio: control 30, in CO 12, in gas 10. Corn seedlings

showed like discrepancies between the normal and the plants grown in CO and gas atmospheres. The ratios of the length of the seedling from the seed to the tip of the longest leaves, taken from three experiments each of which were the averages of at least 8 individuals, is: control 21, in CO 11, in gas 10; of the actual main axis itself: control 20, in CO 15, in gas 10; and of the primary root: control 40, in CO 15, in gas 10. The controls showed about twice as many unfolded leaves as did the plants grown in CO, while in gas the leaves had barely begun to break through their sheaths.

Buckwheat, sunflower and mustard seedlings exhibited similar behavior and the ratios of the length of their hypocotyledonary stems and of their primary roots would be nearly the same as the forms given above. Squash seedlings showed especially well-marked differences in the different gases, the caulicles of the controls being seven times longer than those in gas, while the roots of the former were ten times longer. Those in CO, though a little more developed than the ones in gas, were dwarfed as compared with the normal. Naturally the squash is sensitive to diminished light so that some etiolation was to be expected, but all were under equal light conditions. Perhaps the most striking of all the experiments were those with wheat and rice. The wheat grew but little in gas, but fared better in CO. As to the length of the shoot to the tip of the longest leaf, we have the following ratios: control 12, in CO 9, in gas 1; while on comparison of the average length of the several adventitious roots the difference is not so marked: control 2, in CO 1.2, in gas 1. The difference in the roots became evident on examining the branches; secondary rootlets were almost absent in the specimens from the gas, and were few in those plants grown in CO, but were not only plentifully developed but also branched again in the control experiment. The normal also showed two unfolded leaves with a third just unrolling, while in the CO specimens there was barely one, and in those from gas the leaves had hardly broken out of the curiously swollen leaf-sheath. Rice exhibited one of the most prettily graded series. It grew luxuriantly in the moist atmosphere, and the length of the leaf-tip from the seed being 1 in the gas culture, that in CO would be about 2 and the control 6, with a nearly similar ratio as regards

the average length of the several roots. As to the branching of the roots and the unrolling of the leaves, what was said about the wheat applies here.

In considering the secondary members both of the root and of the shoot, it may be said in general that the seedlings in gas and CO presented a very imperfect development. Branches of the shoot were only observed in *Vicia* and while the control showed such branches frequently the others did not. Secondary roots were observed in all cases, however, and it was found that in CO and gas the formation of the regular root-system was greatly retarded if not inhibited. In the latter secondary branches were few and poorly developed, while the controls would have a healthily developed system. In gas especially the roots often appeared sickly, more so indeed than the shoots. In the corn, while the normal root-system was far from being completely formed in CO or in gas, an interesting exception was seen as to the production of lateral members, which as has been said are usually fewer in these cases than in the normal. Below the circle of adventitious roots ordinarily developed in corn seedlings a considerable number of supernumerary secondary roots arose without order and grew out for a millimeter or two.

In connection with this may be introduced a description of the peculiar thickening noticed at the base of the stem of many of the seedlings grown in CO or in gas. In *Vicia* for instance the normal diameter of the base of the stem next the seed being 3.5 mm., the CO specimens were 4.5 mm., and those in gas were 5.0 mm. in diameter. *Helianthus*, *Fagopyrum* and *Sinapis* also showed a similar but less well-marked thickening, and the curious enlargement of the leaf-sheath in the wheat and rice seedlings has already been referred to. In corn, however, the difference in size of the stem-base is most conspicuous and seems to be connected with the formation of the supernumerary roots just spoken of. The corn is the only form that has so far been examined microscopically and the anatomical conditions are briefly as follows. The cells of the cortical parenchyma are greatly enlarged, though apparently no more in number. In the plants grown in CO these cells measure from one third to one half more in diameter than do those of the control, while in the gas speci-

mens they are swollen to nearly twice their ordinary diameter. The bundle-ring of the developing plerome cylinder is scarcely greater in diameter in the CO specimens than it is in the normal and in the sections examined shows little or no difference in structure. Not so, however, in the plants from the gas cultures. Here the axial bundle-strand is nearly twice the normal diameter, the size of all of the developing bundle-elements is exaggerated to about the same extent as is the parenchyma, and the walls are much less thickened than they normally are. The difference is very striking. The whole stem-base appears to be in a less differentiated condition than normal to it at that age, a circumstance which perhaps accounts for the very free formation of the extra roots.

The normal growth being in general checked by the gases used, it is natural to suppose that the growth-curvatures would also be affected, and such appears to be the case. In CO the seedlings most sensitive (*e. g.*, mustard) showed little or no curvature in response to phototropic stimulus, and in gas whatever development took place was quite irrespective of the angle of the incident rays. Similarly there seemed to be a less degree of sensibility to geotropic stimulus, especially noticeable in the adventitious roots of the corn and in those supernumerary roots formed in the thickened stem of the same plant.

Together with the other inhibitive effects of CO and gas it was noticed that the chlorophyl formed much less quickly than under normal conditions. In fact mustard seedlings, sprouted in the dark, scarcely greened after a week's exposure to sunlight while in an atmosphere with gas, though the control showed the effects of the light in the course of a few hours. In CO the action was also retarded, but not nearly so much as in gas. It must not be supposed, however, that this lack of chlorophyl is necessarily the explanation of the retarded growth. Most of the seedlings in the above described experiments did not in the course of the experiment pass beyond the stage where they chiefly rely upon the stored food in the seed. Also in some cases the chlorophyl did finally assume its normal color, without bringing about any increase in the rate of growth. Finally it may be said that some experiments which were kept in the dark all the time, showed that the etiolated plants behave similarly to the ones in the light.

Effect of nitrous oxide. — N_2O was prepared from NH_4NO_3, and purified over ferrous sulphate, potassic hydrate and water. Seedlings of mustard were arranged in the bell-jars in the same manner as in the CO experiments, except that a mercury seal was used. At the beginning of the experiment the hypocotyledonary stems of the seedlings were on the average 4 mm. long. After five days those of the control were 5 cm. and of the ones in 80 per cent. N_2O, 2.6 cm., while the average of those in 40 per cent. N_2O was about half-way between the two. The root-system also showed the effect of the gas, being both shorter and less branched in the seedlings exposed to the N_2O.

A trial experiment was also made with fungi to see if these plants could use the gas as a source of nitrogen. Three parallel series were set up, one with a normal culture fluid containing a nitrate and two with the same culture fluid minus the nitrate. Of the last two, one was left in air, the other exposed to an atmosphere containing about 60 per cent. N_2O, and normal oxygen. Up to this time neither of the series without nitrates have shown any development, while the control with the normal culture solution has produced a thick fruiting felt. The fungi used were *Sterigmatocystis nigra*, *Penicillium glaucum* and *Rhizopus nigricans*. It has, indeed, been reported somewhat indecisively that nitrous oxide can serve as a nitrogen source, but these negative experiments do not agree with such a conclusion.

Another phase of the influence of the gases was to be found in their action upon the shoots and roots of woody or succulent perennials. If the basal portion of a shoot of such plants as *Gossypium* or *Haematoxylon* were enclosed in a bell-jar with the stem extruding through a tubulure properly sealed around it, both illuminating gas and carbon monoxide acted as a very slow poison which caused the leaves to become desiccated and to fall off after periods varying from about four to twenty days, and the plants were killed.

If entire plants of *Haematoxylon*, *Mimosa*, *Meibomia* (*Desmodium*), *Opuntia* or *Mesembryanthemum*, were enclosed in the bell-jar containing the gases, several important reactions might be noted. First it was to be seen that if the enclosing chambers were filled with a mixture of carbon monoxide or illuminating gas containing over

90 per cent. of carbon monoxide, practical asphyxiation occurred in *Meibomia*, *Haematoxylon* and *Mimosa*, the leaves becoming discolored and turning brown, but not being cast away, the small amount of oxygen present and the strong toxic action of the other gases coöperating to kill the leaf before separatory layers could be formed.

In all tests in which a supply of oxygen fairly equal to the normal was present, the leaves of the three plants were discolored and cast away within a week, and at the end of that period appeared to be much more seriously damaged than those in higher percentages, which appearance however might be seen to be erroneous on closer examination. Plants exposed to both higher and lower proportions for two weeks revived and sent out new leaves when placed in the propagating houses. Tests with young plants of *Opuntia tuna* and with cuttings of a species of *Mesembryanthemum* showed that the last named is much more readily killed by the gases, the entire plant perishing after exposures for a month to atmospheres containing as much as 25.5 per cent. carbon monoxde.

In marked contrast with the foregoing is the behavior of the gametophytes of some of the mosses. Such forms as *Catherinea angustata*, *Dicranella heterophylla* and *Physcomitrium turbinatum*, were able to endure atmospheres containing the highest practicable proportions of carbon monoxide or of illuminating gas for periods of over three months, with but little damage and that to the older leaves, in which the cell-contents and chloroplasts were variously and deleteriously affected. In two instances sporophytes were formed. During this period the gametophytes had formed numbers of new leaves, and their resistance could not be attributed to the possibility of being in a resting condition.

Some observations on a more delicate moss, presumably *Mnium undulatum*, show that these gases are not always without effect even on these plants. It should be said, however, that the changes in the cell-contents herein noted proceed so far and no farther, without involving the death of the plant as a whole.

The *Mnium* leaves after exposure for about three weeks to atmospheres of 80 per cent. CO and of 80 per cent. gas respectively, were in general lighter in color. Microscopical examination con-

firmed this and revealed the fact that the chloroplasts had been considerably affected. In the control the cells were full of large, somewhat elliptical chloroplasts and about 2 per cent. of the cells appeared to be dead. In CO the chloroplasts were smaller, with considerable spaces between them, and were fewer in number. In shape they were usually similar to the normal, though in some cases they were curiously elongated. About 20 per cent. of the cells seemed to be dead. In gas as many as 33 per cent. of the cells were dead and often quite empty, and the chloroplasts were much fewer, paler and smaller than in the control, being often nearly spherical in shape. Some of the specimens were returned to the respective gases in which they had been, some were returned to the air. After two weeks the specimens returned to the air were examined. The cells not actually killed, had in both the CO and the gas specimens almost regained their normal appearance, though still somewhat paler than the normal. In the gas specimen, however, so many of the cells had been killed that the older leaves dried up. The growing tips of all appeared fairly healthy. The plants returned to the gases were examined after an additional five week's interval and very little difference was seen as compared with the condition presented at the first examination.

Philotria Canadensis (*Elodea Canadensis*) was exposed to the same conditions, being arranged so that it came in contact with the moist surrounding atmosphere. The leaves showed a somewhat similar degeneration, but rather more serious. After eight days exposure an examination of the CO experiment demonstrated that while the young leaves were not so much affected as compared with the normal, the older leaves were. Many cells in the latter had their contents plasmolyzed or completely disorganized. In the specimens from the gas more than half of the cells were dead and in those that were not the chloroplasts were small and pale. The occurrence of large oxalate crystals was common, suggesting a derangement of the assimilatory functions. In the control almost all of the cells appeared healthy and showed rotation.

Some specimens of a *Nitella* were followed in a control and an 80 per cent. CO experiment. The older cells of both were attacked by bacteria and killed, but the younger cells remained healthy. In the control the typical rotation was observed and

the cells appeared quite normal. In CO the younger cells while alive were paler than the normal and the peripheral layer of motionless protoplasm was enormously thick and apparently very much denser than in the usual condition. The chloroplasts were gorged with starch.

As a result then of progress already made in our experiments it may be said definitely that carbon monoxide, which has been hitherto considered neutral and without influence on plants, is in effect highly toxic when used to replace, partially or entirely, the nitrogen of the atmosphere. The experiments have not yet yielded sufficient detail to allow any determination of the point at which it ceases to be toxic, or to determine whether or not, like carbon dioxide, it stimulates growth when present in small proportions in the atmosphere. It has been shown, however, that a wide range of reaction to this substance is to be found among plants.

The deleterious action of the carbon monoxide results in modifications, of the rate and amount of growth, of the differentiations of the primary tissues, and of the formation of chlorophyl.

Illuminating gas, containing proportions of carbon monoxide slightly above and below 40 per cent., affords, in addition to the toxic action of the carbon monoxide, the results of the action of other substances deleterious to the plant.

The contents of this abstract will be published as soon as practicable, but the description of the details of the experimental tests and a consideration of the whole subject will await the conclusion of the investigation.